

## INTERNATIONAL COOPERATION TREATY

PCT

## NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and  
Administrative Instructions, Sections 402 and 409)

From the INTERNATIONAL BUREAU

To:

MEYERS, Hans-Wilhelm  
von Kreisler Selting Werner  
P.O. Box 10 22 41  
D-50462 Cologne  
ALLEMAGNE

Date of mailing (day/month/year) 09 July 1999 (09.07.99)	
Applicant's or agent's file reference 990313woMegn	<b>IMPORTANT NOTIFICATION</b>
International application No. PCT/EP99/01391	International filing date (day/month/year) 27 February 1999 (27.02.99)
Applicant BIA SEPARATIONS D.O.O. et al	

The applicant is hereby **notified** of the following in respect of the priority claim(s) made in the international application.

1. ☐ **Correction of priority claim.** In accordance with the applicant's notice received on: ,  
the following priority claim has been corrected to read as follows:
- ☐ even though the indication of the number of the earlier application is missing.
- ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:
2. ☒ **Addition of priority claim.** In accordance with the applicant's notice received on: 12 June 1999 (12.06.99),  
the following priority claim has been added:  
SI 14 July 1998 (14.07.98) P-9800201
- ☐ even though the indication of the number of the earlier application is missing.
- ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:
3. ☐ As a **result of the correction and/or addition** of (a) priority claim(s) under items 1 and/or 2, the (earliest) priority date is:
4. ☐ **Priority claim considered not to have been made.**
- ☐ The applicant failed to respond to the invitation under Rule 26bis.2(a) (Form PCT/IB/316) within the prescribed time limit.
- ☐ The applicant's notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a).
- ☐ The applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.
- The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim. See Rule 26bis.2(c) and the PCT Applicant's Guide, Volume I, Annex B2(1B).
5. ☐ In case where **multiple priorities** have been claimed, the above item(s) relate to the following priority claim(s):
6. A copy of this notification has been sent to the receiving Office and
- ☒ to the International Searching Authority (where the international search report has not yet been issued);
- ☒ the designated Offices (which have already been notified of the receipt of the record copy).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Leitao
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C. 20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 27 September 1999 (27.09.99)	
<b>International application No.</b> PCT/EP99/01391	<b>Applicant's or agent's file reference</b> 990313woMegn
<b>International filing date</b> (day/month/year) 27 February 1999 (27.02.99)	<b>Priority date</b> (day/month/year) 27 February 1998 (27.02.98)
<b>Applicant</b> PODGORNIK, Ales et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
 26 August 1999 (26.08.99)

☐ in a notice effecting later election filed with the International Bureau on:  
 \_\_\_\_\_

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41 22) 740.14.35	Authorized officer Jean-Marie McAdams Telephone No.: (41 22) 338.83.38
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# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

# PCT

To:  
**VON KREISLER, SELTING & WERNER**  
 Attn. Meyers, H.  
 Postfach 10 22 41  
 D-50462 Köln  
 GERMANY

NOTIFICATION OF TRANSMITTAL OF  
 THE INTERNATIONAL SEARCH REPORT  
 OR THE DECLARATION

(PCT-Rule 44.1)

Av	Sg	W	Da	Hi	HP	ME	TW	K
23. AUG. 1999								

Date of mailing  
 (day/month/year)

20/08/1999

Applicant's or agent's file reference

990313woMegn

**FOR FURTHER ACTION**

See paragraphs 1 and 4 below

International application No.

PCT/EP 99/ 01391

International filing date

(day/month/year)

27/02/1999

Applicant

BIA SEPARATIONS D.O.O. et al.

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
 34, chemin des Colombettes  
 1211 Geneva 20, Switzerland  
 Facsimile No.: (41-22) 740.14.35

**For more detailed instructions,** see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest: the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5818 Patentlaan 2  
 NL-2280 HV Rijswijk  
 Tel (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Johannes Hijzelendoorn

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

## INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

### What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

### When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

### Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

### How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

### What documents must/may accompany the amendments?

#### Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:  
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:  
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:  
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or  
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:  
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

**"Statement under article 19(1)" (Rule 46.4)**

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

**It must be in the language in which the international application is to be published.**

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

**Consequence if a demand for international preliminary examination has already been filed**

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

**Consequence with regard to translation of the international application for entry into the national phase**

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

# PCT COOPERATION TREATY

WO 99/44053  
PCT/EP99/01391

**PCT**

## NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

MEYERS, Hans-Wilhelm  
von Kreisler Selting Werner  
P.O. Box 10 22 41  
D-50462 Cologne  
ALLEMAGNE

W	Sg	W	Da	H	HP	M	TW	JH	K
10. SEP 1999									
F 77 X 00177.6 004									

*L. Henry*

<b>Date of mailing (day/month/year)</b> 02 September 1999 (02.09.99)		
<b>Applicant's or agent's file reference</b> 990313woMegn		
<b>IMPORTANT NOTICE</b>		
<b>International application No.</b> PCT/EP99/01391	<b>International filing date (day/month/year)</b> 27 February 1999 (27.02.99)	<b>Priority date (day/month/year)</b> 27 February 1998 (27.02.98)
<b>Applicant</b> BIA SEPARATIONS D.O.O. et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:  
**AU,CN,EP,IL,JP,KP,KR,US**

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

**AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU, ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD, SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW**

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 02 September 1999 (02.09.99) under No. WO 99/44053

### REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent international Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19 month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

### REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No. (41-22) 740.14.35	Authorized officer  <p style="text-align: center;">J. Zahra</p> Telephone No. (41-22) 338.83.38
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**NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF  
THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES**

<b>Date of mailing (day/month/year)</b> 02 September 1999 (02.09.99)	<b>IMPORTANT NOTICE</b>
<b>Applicant's or agent's file reference</b> 990313woMegn	<b>International application No.</b> PCT/EP99/01391

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

## TENT COOPERATION TRE Y

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

MEYERS, Hans-Wilhelm  
von Kreisler Selting Werner  
P.O. Box 10 22 41  
D-50462 Cologne  
ALLEMAGNE

Date of mailing (day/month/year) 09 July 1999 (09.07.99)	<b>IMPORTANT NOTIFICATION</b>
Applicant's or agent's file reference 990313woMegn	
International application No. PCT/EP99/01391	
International publication date (day/month/year) Not yet published	
Applicant BIA SEPARATIONS D.O.O. et al	International filing date (day/month/year) 27 February 1999 (27.02.99) Priority date (day/month/year) 27 February 1998 (27.02.98)

- The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
- An asterisk(\*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
27 Febr 1998 (27.02.98)	P-9800058	SI	28 June 1999 (28.06.99)
14 July 1998 (14.07.98)	P-9800201	SI	28 June 1999 (28.06.99)

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

J. Leitao

Telephone No. (41-22) 338.83.38



# PATENT COOPERATION TREATY

Avk Sg W Da Hi HPJ ME TW JH KI

31. MAI 2000

PCT

K F 22.08.00 / 27.06.00

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Meyers, H.  
VON KREISLER, SELTING & WERNER  
Postfach 10 22 41  
D-50462 Köln  
ALLEMAGNE

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)

Date of mailing  
(day/month/year) 29.05.2000

Applicant's or agent's file reference  
990313woMegn

## IMPORTANT NOTIFICATION

International application No.  
PCT/EP99/01391

International filing date (day/month/year)  
27/02/1999

Priority date (day/month/year)  
27/02/1998

Applicant  
BIA SEPARATIONS D.O.O. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

### 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office  
D-80298 Munich  
Tel +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized officer

Conner, M

Tel. +49 89 2399-2241



## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 990313woMegn	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 99/ 01391	International filing date (day/month/year) 27/02/1999	(Earliest) Priority Date (day/month/year) 27/02/1998
Applicant  BIA SEPARATIONS D.O.O. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

## 1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows.

CHROMATOGRAPHIC DEVICE

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1  
☐ None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No

EP 99/01391

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N30/60 G01N30/48 G01N30/56

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	WO 96 06158 A (JOSIC DJURO) 29 February 1996 (1996-02-29) cited in the application page 10, paragraph 2 - page 11, paragraph 2	1,17-20
A	DE 41 19 203 A (BIO RAD LABORATORIES) 13 February 1992 (1992-02-13) column 8, line 21-43	1,17-20
A	US 4 895 806 A (SANDERSON JAMES A ET AL) 23 January 1990 (1990-01-23) column 5, line 11-27; figures 3,4	1,17-20
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex

## Special categories of cited documents

- A document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

13 August 1999

Date of mailing of the international search report

20/08/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax (+31-70) 340-3016

Authorized officer

Zinngrebe, U

## INTERNATIONAL SEARCH REPORT

International Application No

EP 99/01391

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>CABRERA K ET AL: "SILICAROD TM - A NEW CHALLENGE IN FAST HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS" TRAC, TRENDS IN ANALYTICAL CHEMISTRY, vol. 17, no. 1, 1998, pages 50-53, XP000669181 ISSN: 0165-9936 abstract</p> <p>-----</p>	1, 17-20

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/01391

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 990313woMegn	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP99/01391	International filing date (day/month/year) 27/02/1999	Priority date (day/month/year) 27/02/1998
International Patent Classification (IPC) or national classification and IPC G01N30/60		
Applicant BIA SEPARATIONS D.O.O. et al.		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the report</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2), with regard to novelty, inventive step or industrial applicability citations and explanations supporting such statement</li> <li>VI <input type="checkbox"/> Certain documents cited</li> <li>VII <input checked="" type="checkbox"/> Certain defects in the international application</li> <li>VIII <input checked="" type="checkbox"/> Certain observations on the international application</li> </ul>

Date of submission of the demand 26/08/1999	Date of completion of this report 29.05.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Oechsner de Coninck Telephone No. +49 89 2399 2379 

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP99/01391

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-25 as originally filed

**Claims, No.:**

1-20 as originally filed

**Drawings, sheets:**

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY  
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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims 1-16
	No: Claims 17-20
Inventive step (IS)	Yes: Claims
	No: Claims 1-16
Industrial applicability (IA)	Yes: Claims 1-20
	No: Claims

**2. Citations and explanations**

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**



**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**V.1. Novelty of claims 1-16 (Art.33(2) PCT):**

D1-D3 deal with chromatographic units. Since the term "uniform multimodal pore size distribution" is broad and unclear, the subject matter of claims 1 and 10 differs from D1 (Figs 3,4 and related description) and from D3 (Figs.1-3 and related description) only in the claimed feature (I). The subject matter of claims 1,10 is therefore novel over D1 and D3.

D2 discloses only one porous tube (2) and claims 1 and 10 are also new over this prior art document.

**V.2. Lack of novelty of claims 17-19,20 (Art.33(2) PCT):**

V.2.1. Claims 17-19: The features of these claims are well-known in the present technical field of chromatographic units. The subject matter of these claims therefore lacks novelty.

V.2.2. Claim 20: In view of the objection of lack of clarity of claim 20 (see, § VIII.3), the process steps claimed in claim 20 are considered as not novel in view of the general knowledge in the technical field of chromatographic units.

**V.3. Lack of inventive step of claims 1-16 (Art.33(3) PCT):**

According to D1 (col.2, lines 52-54, and Fig.3), and to D3 (Figs.1-3), the microporous membrane which has chemical species at its surface is wound onto a porous core. It results that this obtained membrane has several turns.

The solution proposed in claims 1 and 10 of the present application, i.e., the structure wherein "at least two porous components A and B and the porous component B embraces the porous component A", corresponds to a slight constructional change of the membrane in the form of turns disclosed by D1 and D3 which offers the same advantages. Therefore, this solution does not involve an inventive step.

The additional features of claims 2-9 and 11-16 are also derivable from D1 and/or D2 and for the rest not surprising in the present technical field. They do not amount to an inventive step.

**Re Item VII**

**Certain defects in the international application**

VII.1. The features of claims 1 and 10 are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

VII.2. According to the requirements of Rule 11.13(I) reference signs not appearing in the drawings shall not appear in the description. This requirement is not met in view of the reference sign (72) mentioned in claims 12,13,17 which is not derivable from the Figures 1-8.

**Re Item VIII**

**Certain observations on the international application**

VIII.1. Product claims 1,10,17-19 have been drafted as separate independent claims. Thus, lack of clarity of the claims as a whole arises, since this plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 1-19 do not meet the requirements of Article 6 PCT.

VIII.2. In addition to the objection raised in above § VIII.1, the following objection is raised: The terms "self-supporting structure", "embraces", "component", "uniform multimodal pore size distribution" used in claim 1, "article" used in claim 10, "collecting element" used in claim 19, are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

VIII.3. It is clear from the description on page 5, 1st §, and in particular, lines 2-4; from page 8, lines 1-4; from p.9, last complete §; from the whole description including the examples, that the following features are essential to the definition of the invention:

(1) the subject matter of the present invention relates to the chromatographic unit (30) of Fig.3 having the features of claim 8 and the features indicated in Figs.1 and 2. The "component C" with its central bore is also part of the unit, A,B and C forming the "porous polymer tube" also called "assembly D" in the present application.

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- (2) the term "uniform multimodal pore size distribution" should be clearly defined as it arises from the description
- (3) the vinyl composition of the polymer as claimed in claim 4 and in process claim 20.

Since independent claim 1 does not contain these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

The same objection is raised against independent process claim 20 which does not contain all process steps necessary to produce the product according to the present invention. In particular:

- no step relating to the chemical modification of the pore surface of the two components A and B is mentioned,
- according to the present process steps only one "component" in a tube structure appears to be produced, no indication of two components (tubes) A and B, A being inserted (instead of the unclear term "embraces") in B.
- no indication on the step to obtain the specific pore distribution.

VIII.4. Since the porous components A and B "are both provided with chemical moieties", all passages of the description which appear to exclude or at least mention the presence of these chemical species on both components as alternative should be deleted (see, in particular, p.8, penultimate §; p.18, 1st line of last § in the terms of "If the polymer tube is to be modified with certain chemical compounds.."; p.19, line 10 to p.20, line 11).

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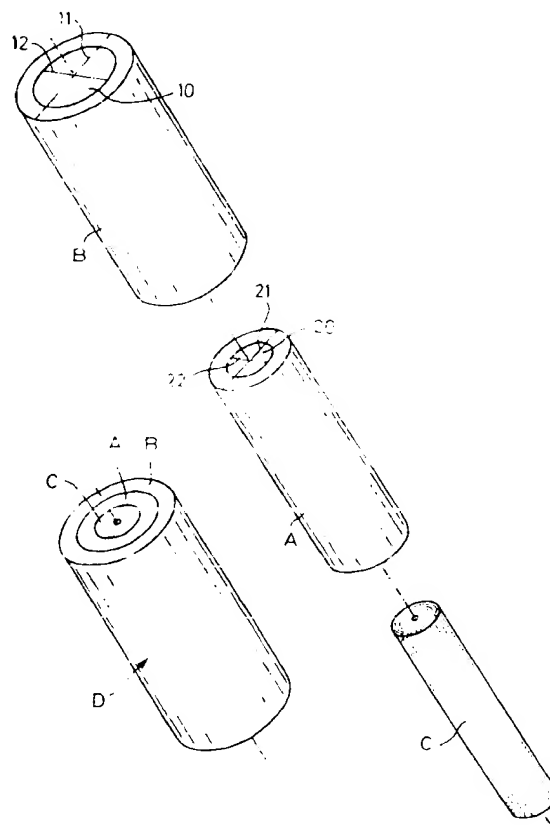
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**(54) Title:** NOVEL CHROMATOGRAPHIC DEVICE

**(57) Abstract**

A porous self-supporting structure comprising at least two porous components A and B and the porous component B embraces the porous component A, wherein (i) surfaces of pores of the at least two porous components A and B are provided with chemical moieties for interaction with substances passing the pores and (ii) the pores of the porous components comprise a uniform multimodal pore size distribution through the entire polymeric structure.



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## Novel chromatographic device

The invention pertains to a porous self-supporting structure comprising at least two porous components A and B, an article comprising a porous self-supporting structure comprising at least two porous components A and B, a housing for use in the article of the invention, end-fittings for use in the article of the invention as well as a process for manufacturing a porous self supporting structure of the present invention.

### BACKGROUND OF THE INVENTION

Liquid chromatography is one of the most important tools for sample analysis as well as for the isolation or purification of compounds. Chromatography works basically via an interaction of molecules dissolved in a liquid phase (mobile phase) and a solid phase (stationary phase). An almost ideal chromatographic process performs an efficient compound separation, sometimes of large sample volume, within very short time. A conventional chromatographic process is carried out by passing a liquid phase containing sample to be separated through a stationary phase (matrix). Since different compounds interact differently with the stationary phase the travelling time is different and as a consequence, a separation occurs. Conventional stationary phases are built in form of porous beads providing a high enough active surface for the interactions. They are packed in columns, normally few centimeters long and few millimetres wide and fixed by porous frits on both ends. Because of their porosity and structure, the beads have rather low mechanical stability. When chromatographic separation systems are scaled up for commercial purposes, more matrix volume is required and thus large columns have to be employed. The combination of high flow rates and larger bed height (i.e. hydrostatic pressure) results in high pressure decrease across the matrix causing compression of the matrix material. This changes the column characteristics due to lower overall porosity and inhomogeneities. One of the attempts to overcome this problem is incorporation of short columns with large cross-sectional area. How-

ever, uneven distribution of the sample over the cross-sectional area and a large dead volume still cause problems. The design of a chromatographic column using horizontal flow solves the problems related to the back pressure by employing a cylinder shaped column as disclosed by Saxena (U.S. Pat. Nos. 4,627,918, 4,676,898 and 4,840,730). The separation matrix is placed between two tube-shaped porous frits of different diameters. The mobile phase passes through the outer porous frit through the matrix. Since the height of the matrix bed is small the hydrostatic pressure does not play an important role. In addition, the bed thickness is low which causes only low back pressures.

Due to the particle structure however, two inherent drawbacks regarding efficiency and speed of the separation remain unsolved: neither the entire bed volume is used for the separation due to the voids (space between the particles) nor the separation time sufficiently short due to diffusion limitations inside one side closed particle pores.

A first attempt to overcome both of the above mentioned problems was introduced by Hjerten et. al J. Chromatogr., 473 (1989) 273-275, WO 90/07965 by polymerising a mixture of acrylic acid and methylenebisacrylamide for the production of a stationary phase. The resulting polymer plug contains channels which are large enough to permit a hydrodynamic flow. The polymer itself is, however, very soft and should be highly compressed before usage. On large scales this leads to a drawback, since the compression produces non-uniform channels within the plug resulting in less than ideal column efficiency. Almost at the same time the so-called „membrane chromatography“ was discovered by Svec et al. (U.S. Pat. Nos- 4,889,632 4,923,610 and 4,952,349). The used membranes have a rigid structure comprising bimodal pore-size distribution of open channels and as such excellent hydrodynamic characteristics resulting in short separation times. Although in principle the size of the membrane is non-limited, mechanical instability and irregular sample distribution limit the application of such units on large scale processes.

Another approach was introduced by Frechet and Svec (US Pat Nos 5,334,310 and 5,453,185) by polymerising monomers to a rigid porous plug within an empty chromatographic steel column of limited diameter. The porous plug has similar characteristics to those of the above mentioned membranes. The high back pressure of the plug however, determines the upper limit of the flow rate which together with a small column diameter prevents applications on preparative level. Josic et. al. disclosed rigid porous tubes based on methacrylates (WO-A-96/06158) where a mobile phase passes the bed in radial direction resulting in much lower back pressure even at elevated flow rates. This design enables a very fast separation on a semi-preparative level.

During the bulk polymerisation of plugs of large diameter or tubes of large thickness a considerable amount of heat is generated. Since the monomer mixture has a relatively low heat conductivity, the temperature within the mixture increases dramatically during the polymerisation (Peters E.C., F. Svec, J.M.J. Frechet, Chem. Mater., 9 (1997) 1898) Since the pore size distribution is temperature dependent (Svec and Frechet, Chem. Mater., 7 (1995) 707), the resulting polymer has a variable structure and cannot be used for good chromatographic separations. Peters et. al., Chem. Mater., 9 (1997) 1898, suggested the polymerisation by slow addition of monomers mixture showing that the temperature increase is much lower and that the pore size distribution is only slightly affected. No separation efficiency of such a column however is presented. In addition, this approach prolongs the time for completion of the polymerisation and requires a very precise addition of the monomer mixture in order to avoid temperature increase.

Another way to control or decrease the amount of heat generated is the addition of polymeric particles of the same pore structure into the monomer mixture. Since the particle diameter is typically in the range of microns, monomers can diffuse into their pores and polymerise resulting in a non-homogeneous pore size distribution. To avoid changes in the polymer structure, pores of the particle should be filled with the in-



hibitor. If the concentration of the particles is too high, the inhibitor inhibits also the polymerisation of monomer mixture around the particles.

On the other hand, if the amount of polymeric particles added to the polymerisation mixture is too low, the particles can settle down in the mould during the polymerisation. In this way the local concentration of the particles in the monomer mixture in the upper part of the mould is low, thus the generated heat is again very large. It is therefore extremely difficult to prepare large porous polymers with a well defined pore size distribution.

Thus, it is an object of the present invention to produce a large scale rigid porous polymer media with well defined uniform pore characteristics.

It is further an object of the present invention to produce large scale rigid porous polymer media exhibiting low back pressures even at high flow rates.

It is still another object of the present invention to produce large scale rigid porous polymer media from large variety of monomers.

It is another object of the present invention to produce large scale rigid porous polymer media in an easy and inexpensive way.

These and further objects of the present invention will be evident from the following description of the present invention as well as from the given examples.

## DISCLOSURE OF THE INVENTION

To achieve foregoing and other objects and in accordance with the purpose of the present invention as embodied and broadly described herein, the present invention is directed to a chromatographic unit comprising a porous polymer tube having a large thickness and housing for the porous polymer tube. The resulting unit can be applied as a chromatographic column, for different bioconversion, adsorption and diagnostic processes as well as a matrix for peptide or oligonucleotide synthesis due to its ability to pass liquids therethrough. The porosity of the porous polymer tube is greater than about 0.2, preferably greater than 0.45. The porosity is defined in terms of water regain or mercury porosimetry.

The material contains small pores i.e. those below 200 nm in diameter, but also large pores of diameter of at least about 700 nm. The porous polymer tube is preferably a cylinder having the inner diameter of at least 1 mm and the outer diameter of at least 10 mm. The porous polymer tube can consist of a single monolith - single monolith porous polymer tube or of a set of tube-shaped monoliths inserted tightly one within another - multi monolith porous polymer tube. Each tube-shaped monolith can have different sorption properties, thus the sorption properties of the porous polymer tube can be tailored according the particular requirements. Also a single monolith porous polymer tube can have different sorption properties due to a two step preparation procedure described herein. The porous polymer tube of the invention is placed in a housing adjusted to the dimensions of the tube. The distributor and collector of the housing are designed to minimise dead volume of the whole unit. The housing can be produced from inert plastic materials e.g. polypropylene or Teflon® or from inert metal like stainless steel.

The porous polymer tube is produced from a mixture of monovinyl monomer and polyvinyl monomer in the presence of a porogen and an initiator. Different mixtures can be used for each tube-shaped monolith to obtain a predetermined characteristic. The predetermined characteristic may be e.g. a non-polar surface of the polymer tube. This can be achieved by introduction of e.g.  $C_4$  or  $C_{18}$  aliphatic groups. Also a polar surface may be desired. In this case different groups like hydroxyl or amino should be present. The thickness of the tube-shaped monolith wall should be established in such a way that during polymerisation the increase of temperature (reaction heat) within the mixture does not exceed the value that affects hydrodynamic characteristics of the final product. The height of the porous polymer tube however, is not limited.

In the case of formation of a multi monolith porous polymer tube, each tube-shaped monolith is polymerised separately in the way that the outer diameter of the inner tube-shaped monolith fits tightly to the inner diameter of the outer tube-shaped monolith. The thickness of the tube-shaped monolith can be different not exceeding however, the critical value affecting the pore structure. In the case of a single monolith porous polymer tube, preferably tube-shaped monoliths having a wall thickness less than critical are polymerised first. The outer diameter of inner tube-shaped monolith is slightly smaller than the inner diameter of the outer tube-shaped monolith. The tube-shaped monoliths are cylindrical and placed one inside the other, the voids in-between are filled with a monomer mixture. The monoliths can be linked together during the polymerisation resulting in a single monolith. After the porous polymer tube is prepared in one or the other way, porogens are washed out with suitable liquid.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Schematic view of the assembly D, the porous polymer tube, of component A, B and a component C

- FIG. 2: Technical drawing of the housing
- FIG. 3: View of the chromatographic unit
- FIG. 4: Second end-fitting
- FIG. 5: A housing according to the invention
- FIG. 6: A component D, the porous polymer tube, of the invention
- FIG. 7: A first end-fitting of the invention
- FIG. 8: A collecting element for reducing the dead volume
- FIG. 9: Comparison of pore size distribution for inner and outer monolith or two-monolith tube
- FIG. 10: Relation between back pressure and a flow rate
- FIG. 11: Separation and purification on multi-monolith tube containing DEAE and affinity active groups
- FIG. 12: Break-through curve of capacity measurement on 50 ml multi-monolith tube

## **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

According to the invention a porous self-supporting structure is provided comprising at least two porous components A and B and the porous component B embraces the porous component A, wherein surfaces of pores of the at least two porous components A and B are provided with chemical moieties for interaction with substances passing the pores and the pores of the porous components comprise a multimodal pore size distribution through the entire polymeric structure.

According to the invention the multimodal pore size distribution (figure 9) means at least three maxima of pore volume over a measured range of from 5 nm to 10  $\mu$ m, which are separated by the regions where the pore volume is low or pores are absent at all. It offers the following advantages:

- Pores with pore diameter above 700 nm exhibit low back pressure at elevate throughput
- Large number of pores with diameter below 700 nm providing high surface area necessary for high binding capacity.

The porous self-supporting structure of the invention comprises a polymer obtainable by polymerisation of monomers having at least two polymerisable moieties or two types of monomers, the first monomer type having one polymerisable moiety and the other monomer type is able to crosslink polymer chains obtained by polymerisation of the first monomer.

In a preferred embodiment of the porous self-supporting structure of the invention the surfaces of the pores are modified with functional groups such as ion-exchange groups, hydrophobic moieties, reactive groups for covalently binding of ligands such as affinity ligands, preferably proteins, enzymes, immunoglobulins, antigens, lectins, sugars, nucleic acids, cell organelles, or dyes, etc.

Preferably, the porous self-supporting structure of the invention utilises polyvinyl monomers and monovinyl monomers for their construction.

In particular, the group of polyvinyl monomers include divinylbenzene, divinylnaphthalene, divinylpyridine, alkylene dimethacrylates, hydroxyalkylene dimethacrylates, hydroxyalkylene diacrylates, oligoethylene glycol diacrylates, vinyl polycarboxylic acids, divinyl ether, pentaerythritol di-, tri-, or tetra methacrylate or acrylate, trimethylolpropane trimethylacrylate or acrylate, alkylene bis acrylamides or methacrylamides, and mixtures thereof.

According to the invention the group of monovinyl monomers include styrene, ring substituted styrenes wherein the substitution include chloromethyl, alkyl with up to 18 carbon atoms, hydroxyl, t-butyloxycarbonyl, halogen, nitro-, amino- groups, protected hydroxyls or amino groups, vinylnaphthalene, acrylates, methacrylates vinylacetate and pyrrolidone, and mixtures thereof.

The polyvinyl monomer or polyvinyl monomer plus the monovinyl monomer are employed in the polymerisation mixture in an amount of 20 to 60% for the production of the porous self-supporting structure of the invention.

The first component B of the porous self-supporting structure of the invention comprises a tube like structure having an inner lumen 10 with an inner diameter 12 and an outer diameter 11 which lumen 10 is able to take up the second component A having an inner lumen 20 with an outer diameter 21 and an inner diameter 22 with the proviso that the outer diameter 21 of component A matches the inner diameter 12 of component B and component A is inserted in component B.

The component A and component B can be of the same or different material, e.g. component A has anion exchange properties and B a reversed phase property.

Preferably, according to the invention the inner lumen 20 of component A serves as a sample collector.

The present invention is concerned also with an article comprising a porous self supporting structure and means for carrying out chromatographic processes. According to the invention the article is preferably a chromatographic unit 30, a column or a cartridge or a bioconversion reactor or matrix for peptide or oligonucleotide synthesis.

A preferred embodiment of the invention comprises a housing 36 providing a sample distributor 23 in which component D is arranged, the housing 36 having at least one inlet 41 and at least one outlet 40, an inner surface 42 and an outer surface 43 and a channel like structure or channel like structures 72 on the central part of its inner surface 42 forming a sample distributor 23, whereas the rest of the inner surface 42 is smooth.

Particularly preferred is a channel like structure 72 expressed as a helical or coiled groove 25 starting at the area of and being in direct contact with the inlet 41 of the chromatographic unit 30 and terminating after at least one complete turn but not in direct connection with the outlet 40 of the chromatographic unit 30. The article of the invention is in particular a chromatographic unit 30 further comprising a second end-fitting 38 and a first end-fitting 32, having O-rings 33,34,35,37 and tightening nuts 31,39.

In a preferred embodiment of the chromatographic unit 30 of the invention the first end-fitting 32 has a top part 62, a bottom part 63 and a casing, the first end-fitting 32 is essentially of cylindrical shape, the first end-fitting 32 comprises a collar 61 dividing the cylindrically shaped end-fitting 32 into two parts 62,63, whereby the part of the end-fitting 32 nearest to the collar 61 is the bottom part 62 comprising a connector

60 in connection with a central bore 64 extending through the entire first end-fitting 32 and an O-ring 35 placed in a ring-like groove in the casing at the area of the top part 63 of the first end-fitting 32 and O-rings in circular grooves in the top part 63 of the first end-fitting 32.

In a preferred embodiment of the chromatographic unit 30 of the invention the second end-fitting 38 has a top part 52, a bottom part 53 and a casing, the second end-fitting 38 is essentially of cylindrical shape, the second end-fitting 38 comprises a collar 51 dividing the cylindrically shaped end-fitting 38 into two parts, whereby the part of the end-fitting 38 nearest to the collar 51 is the top part 52 comprising a connector 50 in connection with a dead-end central bore 54 communicating with a bore 55 which is perpendicular to the dead-end central bore 54, the bore 55 starts in a ring-like groove 56 at the surface of the casing of the second end-fitting 38 and leads into the dead end central bore 54.

A housing 36 for use in the article of claim 13 providing a sample distributor 23 wherein the channel like structure 72 is a helical or coiled groove 25.

As already explained, the component A is inserted into component B. In the case that component A still has a relatively large inner lumen 20 thus would lead to a relatively large dead volume in the whole chromatographic unit. In order to reduce or avoid this drawback, it is possible to insert a collecting element 80 into the component A. The collecting element matches the inner lumen of component A by either fitting tightly into the inner lumen or leaving a gap. When a gap is formed between the collecting element 80 and the inner lumen 20 of component A, this gap is able to work as sample collector and simultaneously rendering the dead volume significantly smaller. In the case that the collecting element fits tightly into component A, of course means must be provided which lead to removal of the liquid running through the porous components A and B. This can be done by providing the outer surface of the collecting element 80



with channels or channel-like structures. Preferably, the channels or channel-like structures 82 are formed by a helical or coiled groove 81. The collecting element having a top part 84 and a bottom part 85 provides on its top part 84 an unstructured surface, preferably a smooth surface. However, the bottom part 85 comprises a channel 83 which extends inwardly from the outer side preferably to the center. The channel-like structure 82 prolongs into the channel 83 on the bottom part 85 of the collecting element 80. Preferably the channel 83 communicates with the bore 64 of the first end-fitting 32. The top part 84 contacts bottom part 53 of the second end-fitting 38.

The chromatographic unit 30 presented herein comprises all parts from figure 2, optionally an element 80 from figure 8 and a porous polymer tube D presented in figures 1 to 6. The housing is preferably made of inert plastic materials e.g. polypropylene or Teflon<sup>®</sup> or from inert metal like stainless steel. Not all the parts have to be made from the same material.

Figure 1 shows an assembly D, the porous polymer tube, of three different components, component A, component B and component C. The three components can be inserted into each other to form a multi monolith porous polymer tube, wherein component C forms the most inner part, component A the middle part and component B the outer part of the concentric assembly D. Component B comprises an inner lumen 10 which diameter 12 is large enough to match the outer diameter 21 of component A. In order to have component C inserted into component A of course the diameter of C has to match the inner diameter 22 of component A. Component C finally comprises a central bore extending through the entire length of component C. The central bore works as sample collector in the assembly D of figure 1. If the central bore has larger diameter an element 80 from figure 8 can be inserted to minimise a dead volume of the collector as well as to provide additional mechanical stability.

Figure 2 shows an exploded view of the chromatographic unit 30 of figure 3. The chromatographic unit consists of tightening nuts 31 and 39 comprising corresponding openings 40 and 41 respectively. The tightening nuts are screwed on the housing 36 in order to keep together the components 32,33,34,35, a multi or single monolith porous polymer tube such as assembly D in the housing 36 as well as the components 37 and 38. Component 32, the first end-fitting, and the second end-fitting 38 is placed at the opposite end. The second end-fitting is inserted into the housing 36 and sealed with the O-ring 37 in the middle part of the fitting 38. The O-ring 37 fits into the groove in the middle of the casing of end-fitting 38. The first end-fitting 32 is inserted into the housing 36 and sealed with the O-rings 33,34 and 35.

Figure 4 shows the second end-fitting 38 having a top part 52 and a bottom part 53 and a casing. The end-fitting 38 is of cylindrical shape and has a collar 51 which divides the cylindrically shaped end-fitting 38 unsymmetrically into two parts. The longer part is inserted into the housing 36 whereby the collar 51 prevents the end-fitting to slide completely into the housing 36. The width of the collar 51 is matching the outer diameter of the housing 36 so that the tightening nut 39 can be screwed over the collar 51 and the housing 36 to fix end-fitting 38. The end-fitting 38 has a central bore which has a dead-end at the lower part of the end-fitting 38 in the area of the bottom part 53. A bore 55 which is perpendicular to the central bore 54 meets the central bore 54. A flow entering the chromatographic unit at the connector 50 runs through the central bore and emerges at the end of the perpendicular bore 55. The perpendicular bore 55 communicates with a groove 56 which is circular-shaped.

Figure 5 shows the situation in the housing. Component D of figures 1 and 6 is placed in the housing 36 in such a way that the bottom part matches the end of distributor 23, but distributor 23 extends over the top of the component D. The position of the component D inside the housing 36 is indicated with dotted lines d1 and d2. The central part of inner wall 42 of the housing 36 shows grooves in a helical or coiled arrange-

ment forming a sample distributor 23. The end-fitting 38 is inserted to touch the component D and communicates with the helical or coiled grooves 25 so that a liquid entering the housing through the bore 55 and the ring groove 56 is lead through the helical or coiled groove 25 at the inner surface 42 of the housing 36 to the outer side of the component D. The O-ring 37 of the end-fitting 38 lies above groove 25 of the distributor 23 and seals the inner surface 42. However, the coiled or helical groove 25 does not communicate with the central bore of the end-fitting 32 shown in figure 7. Therefore, the liquid has to pass the assembly D which is a porous polymer tube. In the central bore of the porous polymer tube working as a collector and optionally comprising the element 80 of figure 8, the liquid is collected and guided to the central bore 64 of the end-fitting 32.

Figure 6 shows schematically a component D, the porous polymer tube, which is placed in figure 5.

Figure 7 shows the first end-fitting 32 which has an analogous shape as the first end-fitting in regard to the collar 61, a casing connector 60 as well as the top part 62 and bottom part 63. However, the central bore 64 extends through the middle of the end-fitting and through its entirety. At the bottom part of the end-fitting 32 O-rings 35,34 and 33 are sealing the end-fitting and the housing 36. The liquid coming from the central bore of the porous polymer tube is guided through the central bore 64 and can be collected.

Figure 8 shows the collecting element 80 having on its outer surface channels or a channel like structure 82 forming a helical or coiled groove collector 81. The top part 84 is smooth, whereas the bottom part 85 comprises a channel 83 extending from the outer side to the center. The channel like structure 82 prolongs into the channel 83 on the lower part 85 of the collecting element 80. The channel 83 communicates with the bore 64 of the first end-fitting 32. The top part 84 touches bottom part 53 of the second end-fitting 38.

The porous polymer tube is produced from a mixture of monovinyl monomer(s) and polyvinyl monomer(s) in the presence of a porogens and optionally an initiator. The polymer contains small pores i.e. those below 200 nm in diameter, but also large pores of diameter at least about 700 nm. The porosity of the polymer is greater than about 0.2, preferably greater than 0.45. Different mixtures can be used for each tube-shaped monolith to obtain desired characteristics. The thickness of the wall of the tube-shaped monolith is preferably established in such a way that during the polymerisation, the increase of the temperature within the mixture does not exceed the value that affects hydrodynamic polymer characteristics negatively. The typical thickness of the tube-shaped monolith obtained in one step polymerisation ranges from few millimetres to few centimetres.

The acceptable temperature range of the polymerisation reaction is determined by performing polymerisation of equal monomer mixtures in a thin sheet-shape mould. The thickness of the mould is preferably such that by measuring the temperature in the middle of the monomer layer during the polymerisation, preferably only a slight or more preferred no temperature increase is detected. Each polymerisation is preferably performed at substantially constant polymerisation temperature, higher of the lowest temperature found to be optimal regarding the sorption properties related to the optimal hydrodynamic characteristics for particular polymer. The hydrodynamic characteristics of the polymer are determined by measurement of pore size distribution with mercury porosimetry, porosity again by mercury porosimetry or by water regain and by measurement of back pressure vs. flow rate. The highest temperature where the characteristics remain unchanged is considered as the upper allowable temperature limit.

The upper value of the thickness of the tube-shaped monolith can be established in a way that tube-shaped moulds of different thickness are prepared and filled with monomer(s) mixture. During the polymerisation, the temperature in the middle of the

monomer(s) mixture layer is recorded. The temperature during the polymerisation should not exceed the value of the upper allowable limit, in order to obtain a monolith suitable for good performing chromatography.

Besides the above mentioned matters, another approach for the determination of tube-shaped monolith thickness is possible. In this case a thick tube-shaped mould is filled with monomer(s) mixture and temperature at different distances from the mould wall is recorded. In this way, a dynamic temperature profile inside the polymerisation mixture is obtained. By deriving equations for particular geometry based on heat balances, the generated specific heat and heat conductivity coefficients can be calculated. Based on this data, a temperature increase inside the tube-shaped mould of defined thickness can be calculated. Furthermore, using the upper allowable temperature limit, a maximal mould thickness can be calculated.

Once the mould thickness for a particular monomer mixture is determined in one or another way, tube-shaped moulds of different inner and outer diameter, but defined difference in-between, can be prepared.

For the preparation of multi monolith porous polymer tube, the tube-shaped monoliths are preferably prepared in a way that the inner porous polymer tube-shaped monolith fits tightly into the outer porous polymer tube-shaped monolith. The height of all tube-shaped monoliths is not limited but is preferably the same. The number of tube-shaped monoliths is basically unlimited and thus multi monolith porous polymer tube of any desired diameters can be obtained.

For the preparation of a single porous polymer tube however, the tube-shaped monoliths are preferably prepared in a way that all are of equal height and the outer diameter of inner porous polymer tube-shaped monolith is smaller than the inner diameter of the outer porous polymer tube-shaped monolith. In this way there is a void space be-

tween the porous polymer tube-shaped monoliths. This space can be filled afterwards with the monomer mixture and polymerisation is performed for the second time or left untreated. The thickness of the void space is again limited by the upper allowable thickness determined for the tube-shaped monoliths. During the polymerisation the different porous polymer tube-shaped monoliths are linked together to form a single porous polymer tube of desired diameter.

Polyvinyl monomers include divinylbenzene, divinylnaphtalene, divinylpyridine, alkylene dimethacrylates, hydroxyalkylene dimethacrylates, hydroxyalkylene diacrylates, oligoethylene glycol diacrylates, vinyl polycarboxylic acids, divinyl ether, pentaerythritol di-, tri-, or tetra methacrylate or acrylate, trimethylolpropane trimethylacrylate or acrylate, alkylene bis acrylamides or methacrylamides, and mixtures of any such suitable polyvinyl monomers.

Monovinyl monomers include styrene, ring substituted styrenes wherein the substitution include chloromethyl, alkyl with up to 18 carbon atoms, hydroxyl, t-butyloxycarbonyl, halogen, nitro, amino group, protected hydroxyls or amino groups, vinylnaphtalene, acrylates, methacrylates vinylacetate and pyrrolidone, and a mixture thereof. The polyvinyl monomer or polyvinyl monomer plus the monovinyl monomer are generally present in the polymerisation mixture in an amount of 20 to 60%.

The porogens can be selected from different types of material like aliphatic hydrocarbons, aromatic hydrocarbons, esters, alcohols, ketones, ethers, solution of soluble polymers, and mixture thereof.

Soluble polymers may also be added to the monomers. They are dissolved from the polymer after its formation and serve for increase of porosity. If they are present, then preferably in an amount of 10 to 40%.

For polymerisation initiation conventional free-radicals generating initiators can be used such as azo compounds e.g. azobisisobutyronitrile and 2,2'-azo-bis(isobutyramide)dihydrate or peroxides e.g. benzoyl peroxide and dipropylperoxydicarbonate. Different initiators may be used to obtain a different pore structure related to the degradation rate of the initiators. The amount of initiator is typically in the range of from about 0.5 to 4% by weight of the monomers

Before the polymerisation mixture is placed in the mould it is preferably deaerated by means of inert gas like nitrogen or argon to remove or displace as much oxygen as possible. Once it is prepared the mould is preferably sealed to prevent contamination with air.

The polymerisation is e.g. carried out in a conventional manner known to the skilled person, generally at a temperature of from about 50° to 90°C for a period up to 48 hours. Preferably the temperature is accurately regulated to obtain defined pore size distribution all over the polymer in a controlled and reproducible manner.

After the porous polymer tube has been formed, it is washed to remove any porogenic solvent as well as to dissolve any soluble polymers, if present. The type of solvent is not critical. Many different solvents can be used, such as methanol, ethanol, benzene, toluene, acetone or tetrahydrofuran. To remove the porogens and dissolved polymers completely, the washing step should be repeated several times.

If the polymer tube is to be modified with certain functional groups, the polymer can be treated with certain chemical compounds. In the case of glycidyl methacrylate (as one monomer for making the polymer) which contains epoxy groups, the polymer can further react with an oleum 1,4-dioxan mixture to give sulfonic groups (SP), chloroacetic acid to give carboxymethyl groups (CM) or different amines like diethy-

lamine to give N,N-diethylamino-2-hydroxypropyl groups (DEAHP), triethylamin hydrochloride for quaternary trimethylamino groups (Q) or ethylenediamine for introduction of amino groups (EDA). Hydrophobic groups may be introduced using alcohols like sodium ethanolat, butanolat or octanolat. The polymer may also react with affinants for specific binding. Affinants can be, but are not limited to, proteins, enzymes, antibodies, antigens, lectins, sugars, dyes, nucleic acids or cell organelles. The polymers based on other monomers can also be treated in analogous ways which are known to the skilled person.

For the preparation of a multi monolith porous polymer tube, the tube-shaped monoliths are washed to remove the porogenic solvent as well as to dissolve any soluble polymers present. If no further modification of the tube-shaped monoliths is required or all the tube-shaped monoliths should bear the same functional groups the tube-shaped monoliths are inserted one into another to form multi monolith porous polymer tube. The additional functional groups can be introduced using one of above described reagents. Finally, the multi monolith porous polymer tube is placed in a housing. If each or at least two of the tube-shaped monoliths should bear different functional groups, the modification should be performed on each tube-shaped monolith separately and only afterwards, be inserted one into another to form a multi monolith porous polymer tube. Another approach is to modify at first the outer tube-shaped monolith. After the modification reaction is completed the unreacted reagent is washed out and smaller tube-shaped monolith is inserted. The assembly can be placed in a new different reagent. Since the reactive groups of the larger tube-shaped monolith are already converted, the reaction is performed only in the smaller tube-shaped monolith. This procedure can be repeated several times, depending on the number of tube-shaped monoliths.

For the preparation of single monolith porous polymer tube, when no further modification of the tube-shaped monoliths or equal functional groups should be introduced to all the tube-shaped monoliths, they are inserted concentrically in a mould one into



another. A monomer mixture previously deaerated is added to fill all voids between the tube-shaped monoliths except a central bore. The mould is sealed and the polymerisation is performed at the same temperature as the tube-shaped monoliths were prepared. After the polymerisation is completed, the single monolith porous polymer tube is washed to remove any porogenic solvent as well as to dissolve any soluble polymers present. If required, functional groups can be introduced into the polymer using the described reagents supra. Finally, the tube is placed in a housing and the unit is ready to be used.

When a single monolith porous polymer tube with different functional groups is to be prepared, the following ways of preparation are possible. If the required functional groups are part of the monomers and different from the one the tube-shaped monoliths were prepared of, a similar procedure is used as in the case of the preparation of the single monolith porous polymer tube with no further modification. The polymerisation temperature may however be different from the one employed during the tube-shaped monolith preparation. Another approach is used, if the functional groups should be introduced into the polymer tube-shaped monolith. The tube-shaped monoliths should firstly be washed to remove any porogenic solvent as well as to dissolve any soluble polymers present. To each, or a set of tube-shaped monoliths the required functional groups are introduced as described above. The tube-shaped monoliths are dried and emerged into the porogen mixture or inert substance which fills the pores and can easily be removed afterwards. In this way the pores are filled and any additional polymerisation within them is slowed down or even inhibited completely. The tube-shaped monoliths prepared in this way are inserted in the mould and processed according to the procedure described for the preparation of the single monolith porous polymer tube with uniform sorption characteristics. Finally, the tube is washed, placed in a housing and the unit is ready to be used.

The present invention will further be explained by the following non-limiting examples.

## EXAMPLES

Example 1: Preparation of a chromatographic unit comprising multi monolith polymer porous tube

Two stainless steel moulds were prepared. The first mould comprises of two stainless steel tubes, outer of inner diameter 35 mm and inner of outer diameter 17.6 mm. The stainless steel tubes have wall thickness of 1.5 mm and having a very high coefficient of heat conductivity. The smaller tube was inserted in the middle of the larger one. The stainless tube with smaller diameter is longer then the stainless tube with larger diameter in order to allow the flow-through of the thermostated liquid with the purpose of removing generated heat. One nut seals the area between inner and outer tube to form a cylindrical shape void in which monomer mixture is placed. A second nut seals the same area on the upper side. This nut has a septum through which the monomer mixture is injected into the mould and a small hole for air to exit during the loading of the monomer mixture. After filling is completed the hole is sealed to prevent air to enter. The second mould is constructed in the similar way with the inner diameter of outer tube of 17.5 mm and the outer diameter of the inner tube of 1.2 mm. A monomer mixture was prepared by mixing glycidyl methacrylate, ethylene dimethacrylate, cyclohexanol, dodecanol and benzoyl peroxide. The mixture was bubbled with nitrogen for 20 minutes to remove any oxygen present. The mixture was injected into the mould until it was completely filled and the polymerisation was started by placing both moulds into a thermostated water bath. After 16 hours the moulds were removed from the bath, cooled to room temperature and the nuts were removed. The tube-shaped monoliths were taken from the moulds and placed in pure methanol. The methanol was changed several times to remove the porogens. The small tube-shaped monolith was then inserted into the hole of the larger one and placed in the appropriate housing.

### Example 2

The multi monolith porous polymer tube of two tube-shaped monoliths was prepared according to the example 1. For each tube-shaped monolith pore size distribution using mercury porosimetry was measured. Both measurements give very similar multimodal pore size distribution presented in figure 9.

### Example 3

The chromatographic device was prepared according to the example 1. The device was connected to the HPLC preparative system and tested under different flow rates up to 450 ml/min. The device exhibits low back pressure even at elevated flow rates. The relationship between back pressure and flow rate was found to be linear (Figure 10).

### Example 4: Preparation of reverse phase multi-monolith tube

The moulds described in the example 1 were used. A monomer mixture was prepared by mixing glycidyl methacrylate, stearyl methacrylate, ethylene dimethacrylate, cyclohexanol, dodecanol and benzoyl peroxide. The mixture was bubbled with nitrogen for 20 minutes to remove any oxygen present. The mixture was injected into the mould until it was completely filled and the polymerisation was started by placing both moulds into thermostated water bath. After 16 hours the moulds were removed from the bath, cooled to room temperature and the nuts were removed. The polymer cylinders were taken from the moulds and placed in pure methanol. The methanol was changed several times to remove the porogens. The small tube-shaped monolith was then inserted into the hole of the larger one and placed in the appropriate housing. The multi monolith porous polymer tube exhibits strong hydrophobic character.

### Example 5: Preparation of chromatographic unit comprising single monolith polymer porous tube

Two stainless steel moulds similar to the ones in example 1 but with different dimensions of void diameters were used for the polymerisation of two tube-shaped monoliths. The inner diameter of larger tube-shaped monolith was 2 mm larger of the outer diameter of the smaller. The smaller, was removed from the mould. The larger tube-shaped monolith was left in the mould from which the inner stainless tube was removed. The mould was sealed from one side with the nut. The smaller tube-shaped monolith was inserted into the hole of the large one remained in the mould. The mould was sealed also on the other side with the septum through which a deaerated monomers mixture from example 1 was added to fill the void between two monolith cylinders. The mould was placed into thermostated bath for 20 hours. After the polymerisation was completed the single monolith porous polymer tube was removed. The monomers were polymerised mixture between two tube-shaped monoliths and inserted to give a single monolith porous polymer tube.

#### Example 6: Separation on chromatographic unit comprising multi monolith porous polymer tube

The multi monolith porous polymer tube was prepared according to example 1. The housing containing the multi monolith porous polymer tube was filled with pure diethylamine and left to react for 24 hours at the temperature of 30°C. After the reaction was completed, the excess of diethylamine was removed by pumping through the multi monolith porous polymer tube water at the flow rate of 5 ml/min. The tube was finally washed with 20 mM Tris-HCl buffer, pH = 7.4.

A Solution of myoglobin (5 mg/ml), conalbumin (10 mg/ml) and trypsin inhibitor (20 mg/ml) in 20 mM Tris-HCl buffer, pH = 7.4 was injected through the loop of 500  $\mu$ l.

Binding buffer was 20 mM Tris-HCl buffer, pH = 7.4 and eluting buffer was 20 mM Tris HCl buffer + 1M NaCl, pH = 7.4. The following separation method was applied:

45 seconds with 100% buffer A, gradient from 100% to 20% buffer A within 3 minutes. Flow rate was 42 ml/min. The separation was followed on UV spectrophotometer at 280 nm and the respective chromatogram is shown in figure 11. In addition, protein binding capacity was measured. The solution of human serum albumin (11 mg/ml) was dissolved in 20 mM Tris-HCl buffer, pH = 7.4 and pumped through the multi monolith porous polymer tube with the flow rate of 10 ml/min. The capacity estimated from the break-through curve presented on figure 12 was around 1 g of protein.

Example 7: Preparation of chromatographic unit comprising multi monolith porous polymer tube containing different functional groups

Two tube-shaped monoliths were prepared using the moulds and procedure described in example 1. Both were placed into pure methanol. The methanol was changed several times to remove the porogens. Finally, the tubes were placed in the mixture of methanol and water 50:50 and in the distilled water. After the washing step was completed, the larger tube-shaped monolith was placed in pure diethylamine at the temperature of 30°C. After 24 hours the tube was removed and placed in distilled water. The water was changed several times to remove all diethylamine from the pores.

The smaller tube-shaped monolith was placed in the immunoglobulin solution (2 mg/ml of IgG in 0.5 phosphate buffer, pH 8.0) at room temperature. After 24 hours the tube was placed in distilled water which was changed several times, to remove remaining protein. The smaller tube-shaped monolith was inserted into the hole of the large one to form multi monolith porous polymer tube and placed in the housing.

Sample of myoglobin (5 mg/ml), conalbumin (10 mg/ml) trypsin inhibitor (20 mg/ml) and protein A (10 mg/ml) in 20 mM Tris-HCl buffer, pH = 7.4 was injected through the 500 µl sample loop.

Binding buffer was 20 mM Tris-HCl buffer, pH = 7.4, eluting buffer from DEAE groups was 20 mM Tris-HCl buffer + 1M NaCl, pH = 7.4 and eluting buffer from affinity groups was 0.5 M acetic acid, pH = 2.5.

All the proteins adsorb on the outer part of the multi monolith porous polymer tube on DEAE groups in ion-exchange mode. After applying salt gradient described in example 6 all proteins are selectively eluted. Since the IgGs bind selectively only protein A, other proteins are eluted out of the multi monolith porous polymer tube. Protein A however binds to the inner part of the multi monolith porous polymer tube and cannot be eluted with the salt gradient since the bond is based on affinity interaction. The protein A is released with the pH change. Using a multi monolith porous polymer tube containing different active groups, one can perform separation and purification within single run.

Claims

1. A porous self-supporting structure comprising at least two porous components A and B and the porous component B embraces the porous component A, wherein
  - (i) surfaces of pores of the at least two porous components A and B are provided with chemical moieties for interaction with substances passing the pores and
  - (ii) the pores of the porous components comprise a uniform multimodal pore size distribution through the entire polymeric structure.
2. The porous self-supporting structure of claim 1 wherein the structure comprises a polymer obtainable by polymerisation of monomers having at least two polymerisable moieties or two types of monomers the first monomer type having one polymerisable moiety and the other monomer type is able to crosslink polymer chains obtained by polymerisation of the first monomer.
3. The porous self-supporting structure of claim 1, wherein the surfaces of the pores are modified with functional groups such as ion-exchange groups, hydrophobic moieties, reactive groups for covalently binding of ligands such as affinity ligands, preferably proteins, enzymes, immunoglobulins, antigens, lectins, sugars, nucleic acids, cell organelles, or dyes, etc.
4. The porous self-supporting structure of claim 2, wherein the monomers are polyvinyl monomers and monovinyl monomers.

5. The porous self-supporting structure of claim 3, wherein the group of polyvinyl monomers include divinylbenzene, divinylnaphtalene, divinylpyridine, alkylene dimethacrylates, hydroxyalkylene dimethacrylates, hydroxyalkylene diacrylates, oligoethylene glycol diacrylates, vinyl polycarboxylic acids, divinyl ether, pentaerythritol di-, tri-, or tetra methacrylate or acrylate, trimethylolpropane trimethylacrylate or acrylate, alkylene bis acrylamides or methacrylamides, and mixtures thereof.
6. The porous self-supporting structure of claim 3, wherein the group of monovinyl monomers include styrene, ring substituted styrenes wherein the substitution include chloromethyl, alkyl with up to 18 carbon atoms, hydroxyl, t-butylloxycarbonyl, halogen, nitro-, amino groups, protected hydroxyls or amino groups, vinylnaphtalene, acrylates, methacrylates vinylacetate and pyrrolidone, and mixtures thereof.
7. The porous self-supporting structure of claims 4 and/or 5, wherein the polyvinyl monomer or polyvinyl monomer plus the monovinyl monomer are present in the polymerisation mixture in an amount of 20 to 60%.
8. The porous self-supporting structure of claim 1, wherein the first component B comprises a tube like structure having an inner lumen (10) with an inner diameter (12) and an outer diameter (11) which lumen (10) is able to take up the second component A having an inner lumen (20) with an outer diameter (21) and an inner diameter (22) with the proviso that the outer diameter (21) of component A matches the inner diameter (12) of component B and component A is inserted in component B.



9. The porous self-supporting structure of claim 8, wherein the inner lumen (20) of component A is a sample collector.
10. An article comprising a porous self-supporting structure according to any one of the foregoing claims and means for carrying out chromatographic processes.
11. The article according to claim 10, wherein the article is a chromatographic unit (30) column or cartridge or a bioconversion reactor or matrix for peptide or oligonucleotides synthesis.
12. The article of claim 11 comprising a housing (36) providing a sample distributor 23 in which component D is arranged, the housing (36) having at least one inlet (41) and at least one outlet (40), an inner surface (42) and an outer surface (43) and a channel like structure or channel like structures (72) forming a sample distributor (23) on its inner surface (42).
13. The article of claim 12, wherein the channel like structure (72) is a helical groove (25) starting at the area of and being in direct contact with the inlet (41) of the chromatographic unit (30) and terminating after at least one complete turn but not in direct connection with the outlet (40) of the chromatographic unit (30).
14. The article of claims 11 to 13, wherein the chromatographic unit (30) further comprises a first end-fitting (32) and a second end-fitting (38), having O-rings (33,34,35,37) and tightening nuts (31,39).

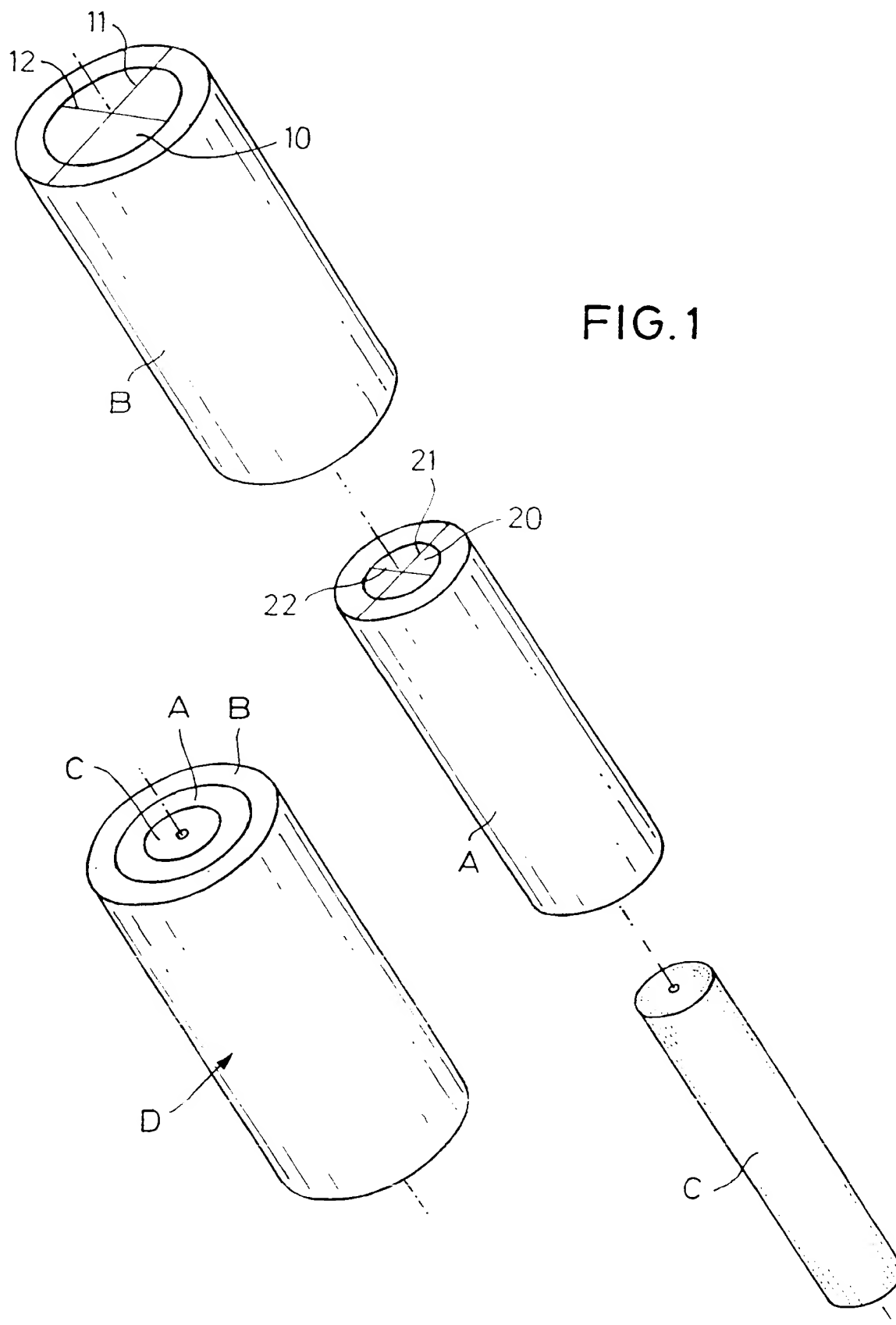
15. The article of claim 14, wherein the second end-fitting (38) has a top part (52), a bottom part (53) and a casing, the second end-fitting (38) is essentially of cylindrical shape, the second end-fitting (38) comprises a collar (51) dividing the cylindrically shaped end-fitting (38) into two parts, whereby the part of the end-fitting (38) nearest to the collar (51) is the top part (52) comprising a connector (50) in connection with a dead-end central bore (54) communicating with a bore (55) which is perpendicular to the dead-end central bore (54), the bore (55) starts in a ring-like groove (56) at the surface of the casing of the second end-fitting (38) and leads into the dead end central bore (54).
16. The article of claim 14, wherein the first end-fitting (32) has a top part (62), a bottom part (63) and a casing, the first end-fitting (32) is essentially of cylindrical shape, the first end-fitting (32) comprises a collar (61) dividing the cylindrically shaped end-fitting (32) into two parts (62,63), whereby the part of the end-fitting (32) nearest to the collar (61) is the bottom part (62) comprising a connector (60) in connection with a central bore (64) extending through the entire first end-fitting (32) and an O-ring (35) placed in a ring-like groove in the casing at the area of the top part (63) of the first end-fitting (32) and O-rings in circular grooves in the top part (63) of the first end-fitting (32).
17. A housing (36) for use in the article of claim 12 providing a sample distributor (23) wherein the channel like structure (72) is a helical groove (25).
18. A first end-fitting (32) or a second end-fitting (38) for use in the article of claim 14.
19. A collecting element (80) for use in the article of claim 10.

20. Process for manufacturing a porous self supporting structure of claim 1 comprising the steps of

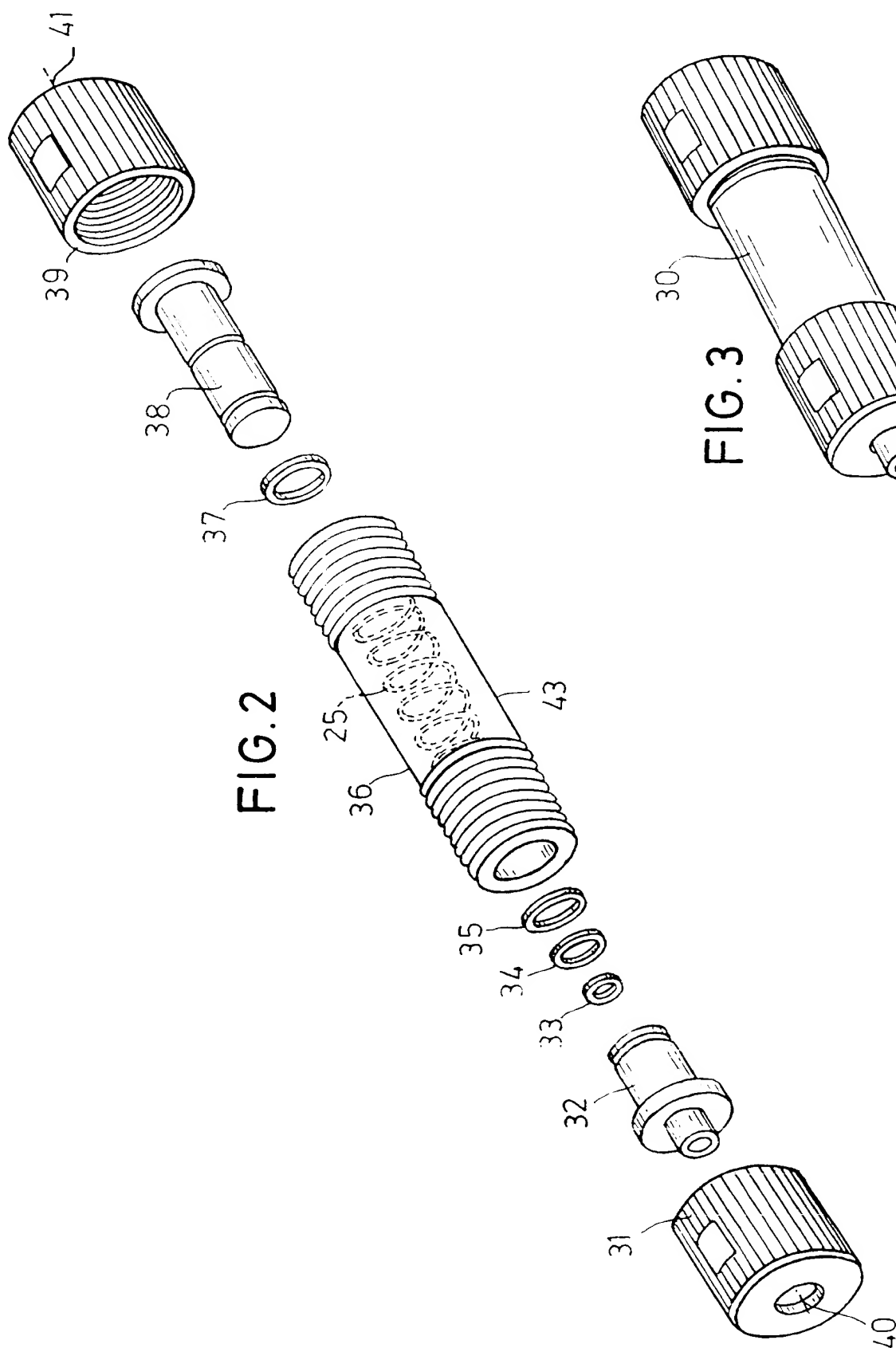
- mixing monovinyl and polyvinyl monomers together with porogens and optionally with polymerisation initiators,
- optionally deaeration,
- pouring the mixture in a mould for casting a tube-like structure,
- controlling the temperature in a range of from 40°C to 90°C,

after formation of the polymer, removing porogens unreacted monomers and initiators or by-products.

- 1 / 5 -



- 2 / 5 -



- 3 / 5 -

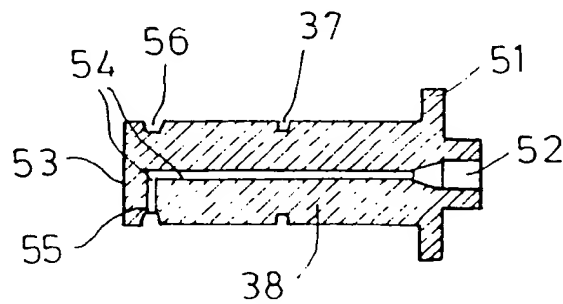


FIG. 4

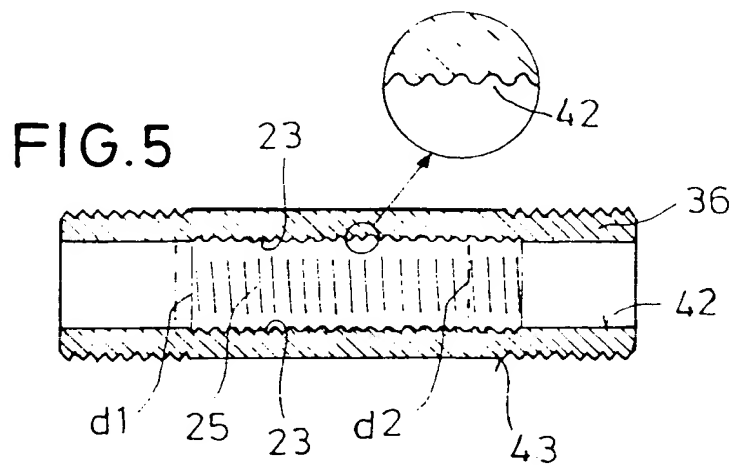


FIG. 5

FIG. 6

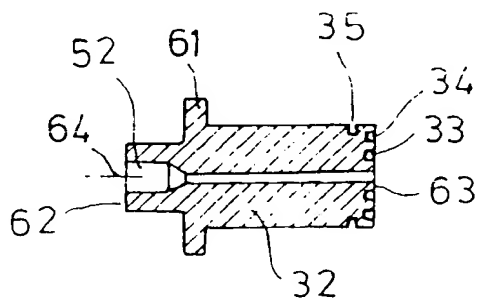
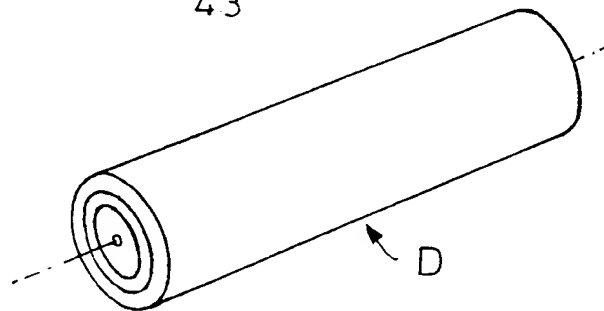


FIG. 7

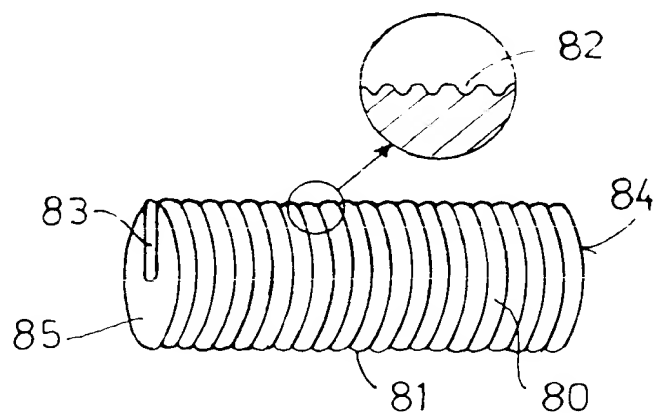


FIG. 8

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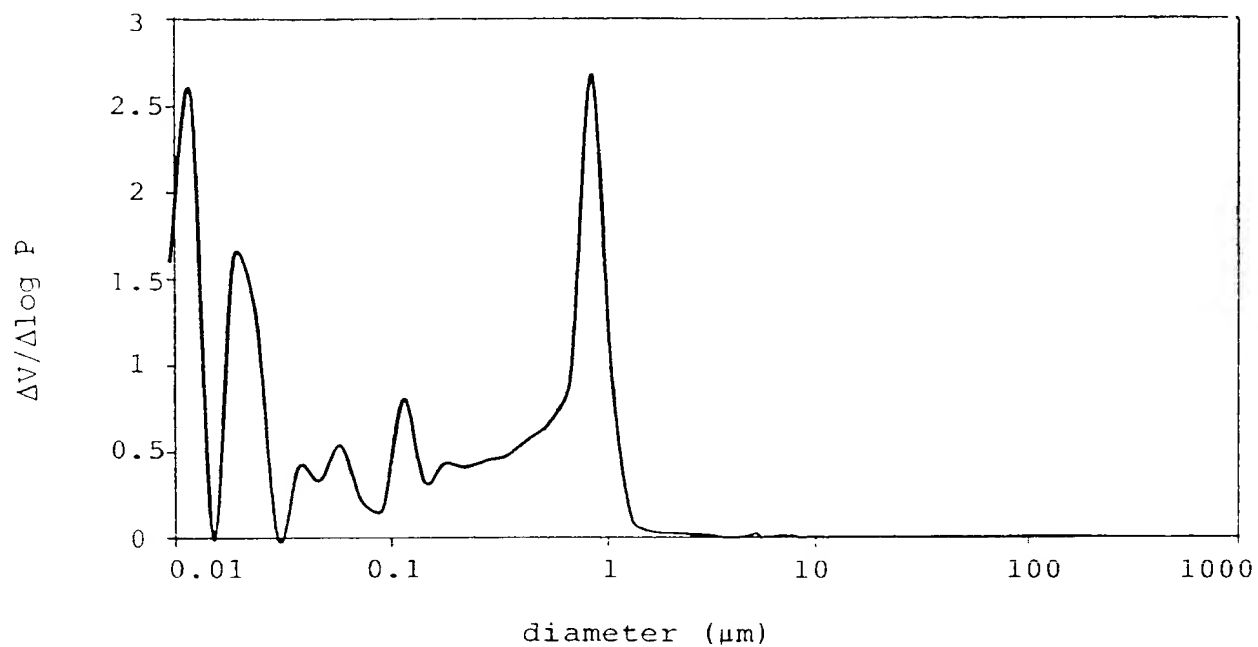


FIGURE 9

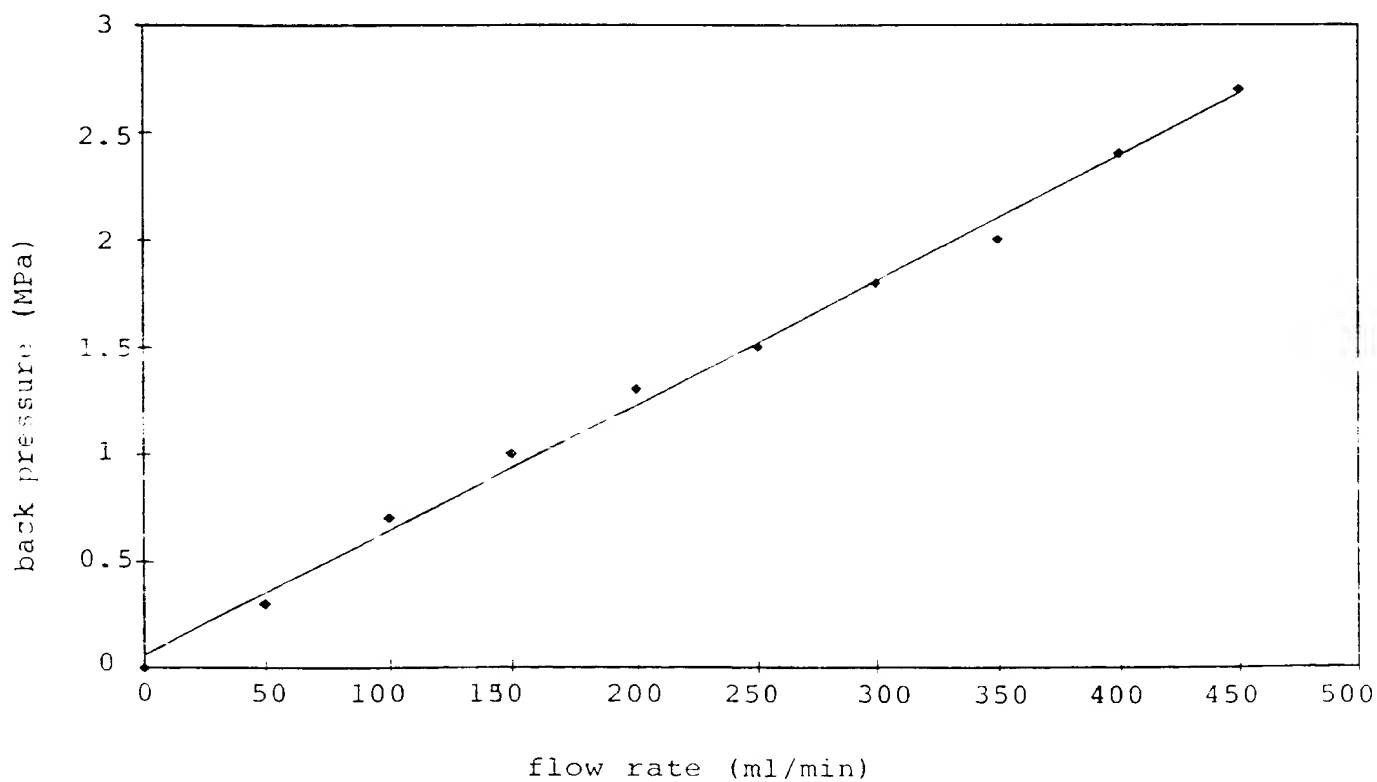


FIGURE 10

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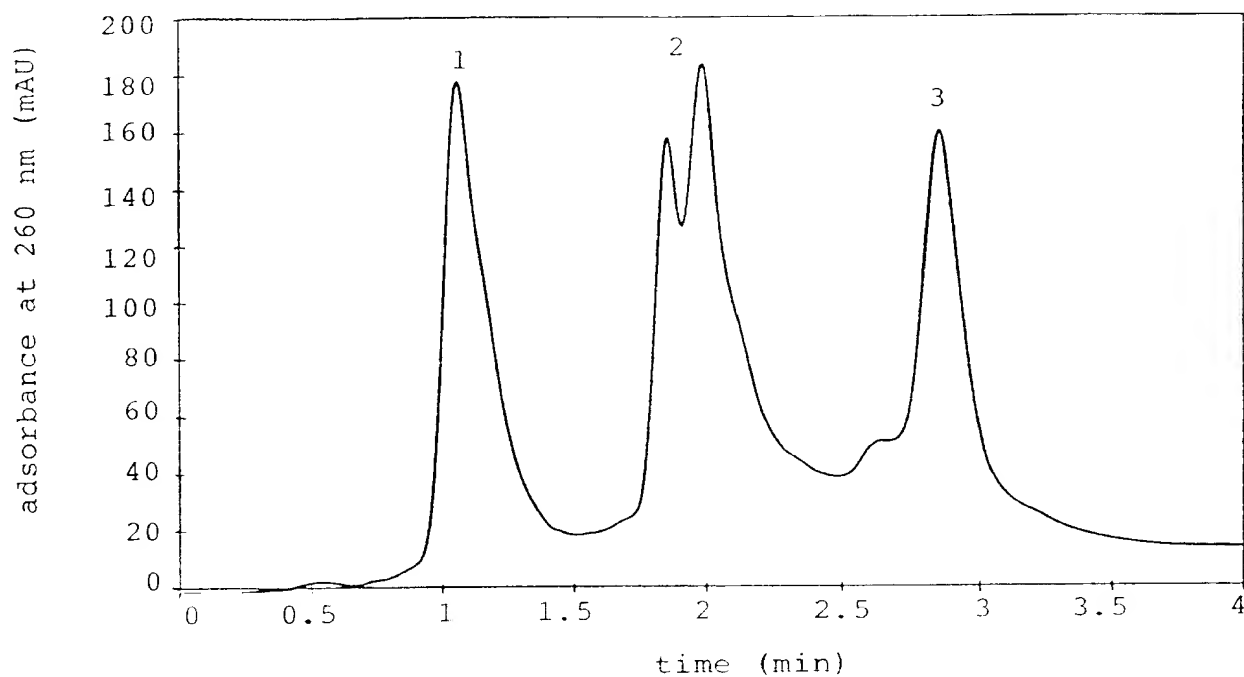


FIGURE 11

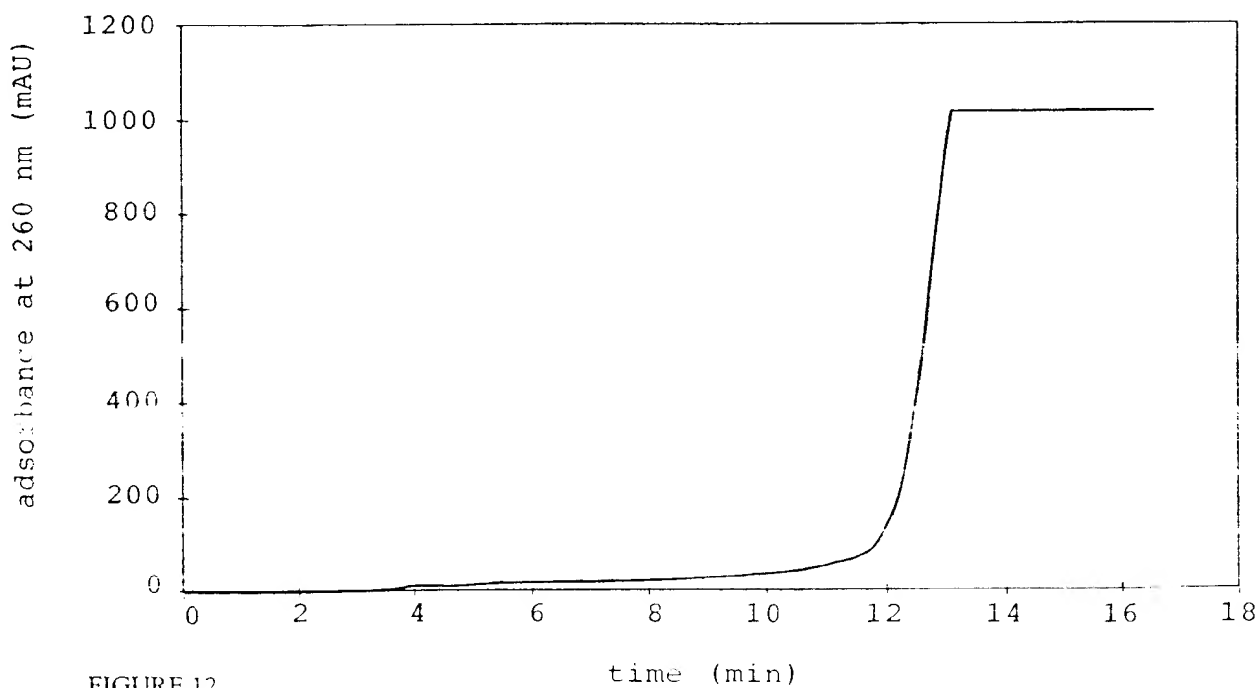


FIGURE 12